1: Protein Expr Purif. 2000 Apr;18(3):388-93. Related Articles, Links

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ELSEVIER SCIENCE FULL-TEXT ARTICLE

Refolding and purification of Zymomonas mobilis levansucrase produced as inclusion bodies in fed-batch culture of recombinant Escherichia coli.

Sunitha K, Chung BH, Jang KH, Song KB, Kim CH, Rhee SK.

Korea Research Institute of Bioscience and Biotechnology, Taejon, Yusong, 305-600, Korea.

PubMed Services

Zymomonas mobilis levansucrase was overproduced by the fed-batch culture of recombinant Escherichia coli harboring a novel expression system that is constitutively expressed by the promoter from the Rahnella aquatilis levansucrase gene. Most of the levansucrase was produced as inclusion bodies in the bacterial cytoplasm, accounting for approximately 20% of the total cellular protein. Refolding after complete denaturation by high concentrations of urea or guanidine hydrochloride was not successful, resulting in large amounts of insoluble aggregates. During the development of the refolding method, it was found that direct solubilization of the inclusion bodies with Triton X-100 reactivated the enzyme, with a considerable refolding efficiency. About 65% of inclusion body levansucrase was refolded into active levansucrase in the renaturation buffer containing 4% (v/v) Triton X-100. The in vitro refolded enzyme was purified to 95% purity by single-step DEAE-Sepharose ion exchange chromatography. Triton X-100 was removed by this ion exchange chromatography.

PMID: 10733894 [PubMed - indexed for MEDLINE]

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L2          156 S L1 AND (LEVAN OR UREAFACIENS OR DIFRUCTOSE DIANYHYDRIDE)
L3           82 S L2 AND (PURIF? OR ISOLAT? OR CHARACT? OR CLON?)
L4            6 S L3 AND (MOBILIS)
L5            2 DUP REM L4 (4 DUPLICATES REMOVED)
L6            6 S L2 AND MOBILIS
L7            2 DUP REM L6 (4 DUPLICATES REMOVED)
L8           35 DUP REM L3 (47 DUPLICATES REMOVED)
L9            2 S L8 AND ZYMOMONAS
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L8 ANSWER 35 OF 35 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 14

ACCESSION NUMBER: 1984:63893 CAPLUS
DOCUMENT NUMBER: 100:63893
TITLE: Action of **levan fructotransferase** of Arthrobacter **ureafaciens** on levanoligosaccharides
AUTHOR(S): Tanaka, Kuniji; Karigane, Takashi; Yamaguchi, Fumio; Nishikawa, Shigeko; Yoshida, Noriko
CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan
SOURCE: Journal of Biochemistry (Tokyo, Japan) (1983), 94(5), 1569-78
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Levan fructotransferase** (I) of A. **ureafaciens**, which produces di-D-fructose 2,6':6,2' dianhydride (difructose anhydride IV) from **levan** by an intramol. transfructosylation reaction, was **purified** to give a single protein band of pI 4.5-4.7 on isoelec. focusing. I had a mol. wt. of 128,000 by gel filtration on Sephadex G-200 and 60,000 on SDS-polyacrylamide disc gel electrophoresis, suggesting that the enzyme is composed of 2 identical subunits. The shortest levanoligosaccharide chain required for difructose anhydride IV formation was detd. to be tetraose. TLC of the enzymic digest of a modified levanhexaose derived from levanhexaose by the redn. of the reducing end to an alditol residue with NaBH4 gave the difructose anhydride IV spot, suggesting that I attacks the modified levanhexaose mol. from the direction of the nonreducing fructose end. Enzymic digests of levantetraose, -pentaose, and -hexaose gave, in addn. to the difructose anhydride IV spot, spots of oligofructans of lower mobility than the original substrate on TLC. From the digest of levantetraose, a hexaoligofructan and a smaller amt. of a pentaoligofructan, but no fructose were sepd., indicating enzymic intermol. levanbiosyl and fructosyl transfer reactions.

=> d l8 ibib ab 30-35

L8 ANSWER 30 OF 35 LIFESCI COPYRIGHT 2003 CSA
ACCESSION NUMBER: 89:92984 LIFESCI
TITLE: Production of a non-reducing fructotrisaccharide from **levan** in the culture of Arthrobacter **ureafaciens** .
AUTHOR: Tanaka, K.
CORPORATE SOURCE: Dep. Nutr., Koshien Univ., Takarazuka, Hyogo 665, Japan
SOURCE: AGRIC. BIOL. CHEM., (1989) vol. 53, no. 8, pp. 2275-2276.
DOCUMENT TYPE: Journal
FILE SEGMENT: J
LANGUAGE: English
AB Formation of a non-reducing fructotrisaccharide, designated La sugar, from **levan** by an action of the **levan fructotransferase** of Arthrobacter **ureafaciens** has been reported. This paper reports its **isolation** and identification.

L8 ANSWER 31 OF 35 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1989-00538 BIOTECHDS
TITLE: A method for preparing inulin-**fructotransferase**; by culturing Pseudomonas fluorescens
PATENT ASSIGNEE: Maruzen-Synth.Chem.
PATENT INFO: JP 38219372 B 13 Sep 1988
APPLICATION INFO: JP 1987-52991 10 Mar 1987
PRIORITY INFO: JP 1987-52991 10 Mar 1987
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1988-297730 [42]
AB A new method for producing inulin-**fructotransferase** (EC-2.4.1.93) comprises culturing Pseudomonas spp., which show enzyme production, in a culture medium containing fructose polymer and recovering the enzyme from the culture broth. Inulin-**fructotransferase** hydrolyzes inulin to di-D-fructosylfuranose-1,2':2,3'-dianhydride, which is a sweetener. The enzyme is produced in higher amounts by Pseudomonas spp. than by Arthrobacter **ureafaciens** and is produced in a more pure form, free from beta-D-fructofuranosidase (EC-3.2.1.26). The produced enzyme is stable and can be stored for several mth in a refrigerator. The preferred bacterium is Pseudomonas fluorescens strain MZ No.949 (FERM P-9235) and is **isolated** from soil. (3pp)

L8 ANSWER 32 OF 35 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1989-03534 BIOTECHDS
TITLE: **Purification** and some properties of inulin-**fructotransferase** (depolymerizing) from Arthrobacter ilicis; **isolation** and **characterization**
AUTHOR: Kawamura M; Takahashi S; Uchiyama T
LOCATION: Department of Biology, Osaka Kyoiku University, Tennoji-ku, Osaka 543, Japan.
SOURCE: Agric.Biol.Chem.; (1988) 52, 12, 3209-10
CODEN: ABCHA6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Inulin-**fructotransferase** (depolymerizing) (EC-2.4.1.93) was produced by Arthrobacter ilicis OKU17B, a soil **isolate**, when grown on inulin culture medium. The enzyme was **purified** from culture supernatant by ionexchange on DEAE-Toyopearl 650M and SP-Toyopearl 650M (twice). The enzyme was **purified** 97-fold, and 14% recovery was achieved. The mol.wt. of the inulin-**fructotransferase** was 50,000, comprising 2 subunits of mol.wt. 27,000. The enzyme had maximum activity at pH 5.5 and 60 deg, and was stable at pH 4-11 and up to 70 deg after incubation at pH 7.0 for 30 min.

Enzyme activity was little affected by various cations at 1 mM (CaCl2, NiCl2, ZnCl2, FeCl3, CuCl2, MnCl2, MgCl2, CoCl2 and HgCl2), differing from the Arthrobacter **ureafaciens** inulin-**fructotransferase** which was inhibited by 1 mM HgCl2 and CuCl2. The principal reaction product after incubation of depolymerizing inulin-**fructotransferase** with inulin was identified as di-D-fructose-2', 1:2, 3'-dianhydride. The dimeric form of the enzyme differed from inulin-**fructotransferases** of other spp., such as Arthrobacter grobiformis C11-1, where the enzyme was monomeric. (10 ref)

L8 ANSWER 33 OF 35 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 12
ACCESSION NUMBER: 1989:3410 CAPLUS
DOCUMENT NUMBER: 110:3410
TITLE: Enzymic formation of di-D-fructose anhydrides from fructan
AUTHOR(S): Uchiyama, Takao; Tanaka, Kuniji; Kawamura, Mishio
CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan
SOURCE: Denpun Kagaku (1988), 35(2), 113-20
CODEN: DPNKAV; ISSN: 0021-5406
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

AB The action of a novel inulin (I)-degrading enzyme, inulin **fructotransferase** (depolymg.) (EC 2.4.1.93), produced by a strain of Arthrobacter **ureafaciens**, is described. The enzyme acts on I to form difructose anhydride (di-O-D-fructofuranose-.alpha.-2,3';.beta.-2;1-dianhydride) (II). II was hydrolyzed by an intracellular enzyme from A. **ureafaciens** and Klebsiella oxytoca at .alpha.-2,3'-links to form inulobiose (1-O-.beta.-D-fructofuranosyl-D-fructofuranose) (III). This enzyme was an .alpha.-fructofuranosidase of a new type. It was **purified** 172-fold from bacterial cells, and had a pH optimum at 7.0 and a mol. wt. of 70,000; it is a dimer consisting of 2 subunits of mol. wt. 36,000. A .beta.-fructofuranosidase which cleaved III was also partially **purified** from A. **ureafaciens**. Pathways of I metab. through difructose dianhydrides were discussed.

L8 ANSWER 34 OF 35 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 13
ACCESSION NUMBER: 1985:500785 CAPLUS
DOCUMENT NUMBER: 103:100785
TITLE: Intermolecular fructosyl and levanbiosyl transfers by **levan fructotransferase** of Arthrobacter **ureafaciens**
AUTHOR(S): Tanaka, Kuniji; Karigane, Takashi; Fujii, Sachie; Chinzaka, Takuma; Nagamura, Shinichi
CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan
SOURCE: Journal of Biochemistry (Tokyo, Japan) (1985), 97(6), 1679-88
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A **purified levan fructotransferase** prepn. from the culture of A. **ureafaciens**, which produces di-D-fructose 2,6':6,2' dianhydride (difructose anhydride IV) from **levan** by an intramol. **levan** fructosyl transfer (ILFT) reaction, produced a trioligofructan and a tetraoligofructan from **levan** in the presence of levanbiose, indicating intermol. fructosyl and levanbiosyl transfer (LFT and LBT, resp.) reactions. The tri- and tetraoligofructans were levantriose and -tetraose, resp. An increase in the levanbiose concn. brought about an increased prodn. of both oligofructans with decreased formation of difructose anhydride IV, supporting the previous theory that the ILFT, LFT, and LBT reactions are catalyzed by the same enzyme. In addn., there existed a roughly stoichiometric relation between the increase and decrease in the prodn. of these oligofructans, and the LBT reaction occurred more intensively than the LFT reaction. The acceptor specificity of the LFT and LBT reactions was studied by using 15

sugars, including mono-, di-, and trisaccharides. The enzyme showed both of the reactions only with levanbiose, -triose, and kestose, indicating that the exposed nonreducing levanbiosyl residue was essential for the acceptor and suggesting the existence of a levanbiosyl acceptor site on the enzyme mol.

L8 ANSWER 35 OF 35 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 14
ACCESSION NUMBER: 1984:63893 CAPLUS
DOCUMENT NUMBER: 100:63893
TITLE: Action of **levan fructotransferase** of Arthrobacter **ureafaciens** on levanoligosaccharides
AUTHOR(S): Tanaka, Kuniji; Karigane, Takashi; Yamaguchi, Fumio; Nishikawa, Shigeko; Yoshida, Noriko
CORPORATE SOURCE: Dep. Biol., Osaka Kyoiku Univ., Osaka, 543, Japan
SOURCE: Journal of Biochemistry (Tokyo, Japan) (1983), 94(5), 1569-78
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Levan fructotransferase** (I) of A. **ureafaciens**, which produces di-D-fructose 2,6':6,2' dianhydride (difructose anhydride IV) from **levan** by an intramol. transfructosylation reaction, was **purified** to give a single protein band of pI 4.5-4.7 on isoelec. focusing. I had a mol. wt. of 128,000 by gel filtration on Sephadex G-200 and 60,000 on SDS-polyacrylamide disc gel electrophoresis, suggesting that the enzyme is composed of 2 identical subunits. The shortest levanoligosaccharide chain required for difructose anhydride IV formation was detd. to be tetraose. TLC of the enzymic digest of a modified levanhexaose derived from levanhexaose by the redn. of the reducing end to an alditol residue with NaBH4 gave the difructose anhydride IV spot, suggesting that I attacks the modified levanhexaose mol. from the direction of the nonreducing fructose end. Enzymic digests of levantetraose, -pentaose, and -hexaose gave, in addn. to the difructose anhydride IV spot, spots of oligofructans of lower mobility than the original substrate on TLC. From the digest of levantetraose, a hexaoligofructan and a smaller amt. of a pentaoligofructan, but no fructose were sepd., indicating enzymic intermol. levanbiosyl and fructosyl transfer reactions.

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☐ 1. Document ID: US 4985365 A

L2: Entry 1 of 10 File: USPT Jan 15, 1991

US-PAT-NO: 4985365
DOCUMENT-IDENTIFIER: US 4985365 A

TITLE: Process for producing optically active benzyl alcohol compound

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: JP 2002017366 A

L2: Entry 2 of 10 File: JPAB Jan 22, 2002

PUB-NO: JP02002017366A
DOCUMENT-IDENTIFIER: JP 2002017366 A
TITLE: NEW LEVAN FRUCTOTRANSFERASE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: JP 11069978 A

L2: Entry 3 of 10 File: JPAB Mar 16, 1999

PUB-NO: JP411069978A
DOCUMENT-IDENTIFIER: JP 11069978 A
TITLE: LEVAN FRUCTOTRANSFERASE GENE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Clip Img | Image

☑ 4. Document ID: JP 01091793 A

L2: Entry 4 of 10 File: JPAB Apr 11, 1989

PUB-NO: JP401091793A
DOCUMENT-IDENTIFIER: JP 01091793 A
TITLE: PRODUCTION OF DI-D-FRUCTOSYLFURANOSE 2,6':6,2'-DIANHYDRIDE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Clip Img | Image

☐ 5. Document ID: JP 01091777 A

L2: Entry 5 of 10 File: JPAB Apr 11, 1989

PUB-NO: JP401091777A
DOCUMENT-IDENTIFIER: JP 01091777 A
TITLE: PRODUCTION OF LEVAN FRUCTOTRANSFERASE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 6. Document ID: JP 2002017366 A

L2: Entry 6 of 10 File: DWPI Jan 22, 2002

DERWENT-ACC-NO: 2002-287313
DERWENT-WEEK: 200233

TITLE: A new levan fructotransferase

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 7. Document ID: JP 2003512045 W WO 200129185 A1 AU 200114197 A KR 2001037651 A EP 1141236 A1 CN 1340097 A

L2: Entry 7 of 10 File: DWPI Apr 2, 2003

DERWENT-ACC-NO: 2001-308483
DERWENT-WEEK: 200325

TITLE: Producing difructose dianhydride IV from sucrose, involves reacting sugar solution in the presence of levansucrase to produce levan, and reacting levan solution in the presence of levan fructotransferase to produce DFA IV

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 8. Document ID: JP 11069978 A

L2: Entry 8 of 10 File: DWPI Mar 16, 1999

DERWENT-ACC-NO: 1999-247463
DERWENT-WEEK: 199921

TITLE: Levan fructotransferase gene - for recombinant production of levan fructotransferase

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 9. Document ID: JP 01091793 A JP 2614460 B2

L2: Entry 9 of 10 File: DWPI Apr 11, 1989

DERWENT-ACC-NO: 1989-148148
DERWENT-WEEK: 199726

TITLE: Di:D-fructosyl-furanose-2,6',6,2'-di: anhydride prepn. - by treating levan with levan fructo-transferase produced by bacterial strain of pseudomonas, for low calorific non-tooth-decaying sugar

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Drawi Desc | Image

☐ 10. Document ID: JP 01091777 A JP 2561100 B2

L2: Entry 10 of 10 File: DWPI Apr 11, 1989

DERWENT-ACC-NO: 1989-148137
DERWENT-WEEK: 199702

TITLE: Levan fructo-transferase prepn. - by culturing Pseudomonas bacteria in culture medium contg. fructose polymer, and recovering prod.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Drawi Desc | Image

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L1 same (levan or mobilis or ureafaciens of difructose dianyhydride)	10

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L1	fructotransferase	29	L1

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